Claims

1. A protein conjugate comprising a physiologically active polypeptide, a non-peptide polymer and an immunoglobulin Fc fragment, which are covalently linked to one.

2. The protein conjugate according to claim 1, wherein the non-peptide polymer is covalently linked via a reactive group at both ends thereof to the physiologically active polypeptide and the immunoglobulin Fc fragment.

3. The protein conjugate according to claim 2, wherein one or more complexes of the physiologically active polypeptide and the non-peptide polymer are covalently linked to a single molecule of the immunoglobulin Fc fragment.

4. The protein conjugate according to claim 1, wherein the immunoglobulin Fc fragment is non-glycosylated.

5. The protein conjugate according to claim 1, wherein the immunoglobulin Fc fragment is composed of one to four domains selected from the group consisting of C_H1, C_H2, C_H3 and C_H4 domains.

6. The protein conjugate according to claim 5, wherein the immunoglobulin Fc fragment further includes a hinge region.

7. The protein conjugate according to claim 1, wherein the immunoglobulin Fc fragment is selected from the group consisting of Fc fragments from IgG, IgA, IgD, IgE, IgM, and combinations and hybrids thereof.

8. The protein conjugate according to claim 7, wherein the immunoglobulin Fc fragment is selected from the group consisting of Fc fragments from IgG1, IgG2, IgG3, IgG4, and combinations and hybrids thereof.

9. The protein conjugate according to claim 8, wherein the immunoglobulin Fc fragment is an IgG4 Fc fragment.

10. The protein conjugate according to claim 9, wherein the immunoglobulin Fc fragment is a human aglycosylated IgG4 Fc fragment.

11. The protein conjugate according to claim 2, wherein the reactive group of the non-peptide polymer is selected from the group consisting of an aldehyde group, a propione aldehyde group, a butyl aldehyde group, a

maleimide group and a succinimide derivative.

12. The protein conjugate according to claim 11, wherein the succinimide derivative is succinimidyl propionate, succinimidyl carboxymethyl, hydroxy succinimidyl or succinimidyl carbonate.

13. The protein conjugate according to claim 12, wherein the non-peptide polymer has a reactive aldehyde group as a reactive group at both ends thereof.

14. The protein conjugate according to claim 1, wherein the non-peptide polymer is linked at each end thereof to a free reactive group at an amino terminal end, a lysine residue, a histidine residue or a cysteine residue of the immunoglobulin Fc fragment and the physiologically active polypeptide.

15. The protein conjugate according to claim 1, wherein the non-peptide polymer is selected from the group consisting of polyethylene glycol single polymers, polypropylene glycol single polymers, ethylene glycol-propylene glycol copolymers, polyoxyethylated polyols, polyvinyl alcohols, polysaccharides, dextrans, polyvinyl ethyl ethers, biodegradable polymers, lipid polymers, chitins, hyaluronic acids, and combinations thereof.

16. The protein conjugate according to claim 15, wherein the non-peptide polymer is polyethylene glycol.

17. The protein conjugate according to claim 1, wherein the physiologically active polypeptide is selected from the group consisting of hormones, cytokines, enzymes, antibodies, growth factors, transcription regulatory factors, coagulation factors, vaccines, structural proteins, ligand proteins and receptors.

18. The protein conjugate according to claim 17, wherein the physiologically active polypeptide is selected from the group consisting of human growth hormone, growth hormone releasing hormone, growth hormone releasing peptide, interferons, interferon receptors, colony stimulating factors, glucagon-like, G-protein-coupled receptor, interleukins, interleukin receptors, enzymes, interleukin binding proteins, cytokine binding proteins, macrophage activating factor, macrophage peptide, B cell factor, T cell factor, protein A, allergy inhibitor, cell necrosis glycoproteins, immunotoxin, lymphotoxin, tumor necrosis factor, tumor suppressors, metastasis growth factor, alpha-1 antitrypsin, albumin, alpha-lactalbumin, apolipoprotein-E, erythropoietin, highly glycosylated erythropoietin, angiopoietins, hemoglobin, thrombin,

thrombin receptor activating peptide, thrombomodulin, factor VII, factor VIIa, factor VIII, factor IX, factor XIII, plasminogen activating factor, fibrin-binding peptide, urokinase, streptokinase, hirudin, protein C, C-reactive protein, renin inhibitor, collagenase inhibitor, superoxide dismutase, leptin, platelet-derived growth factor, epithelial growth factor, epidermal growth factor, angiostatin, angiotensin, bone growth factor, bone stimulating protein, calcitonin, insulin, atriopeptin, cartilage inducing factor, elcatonin, connective tissue activating factor, tissue factor pathway inhibitor, follicle stimulating hormone, luteinizing hormone, luteinizing hormone releasing hormone, nerve growth factors, parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocortical hormone, glucagon, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, autotaxin, lactoferrin, myostatin, receptors, receptor antagonists, cell surface antigens, virus derived vaccine antigens, monoclonal antibodies, polyclonal antibodies, and antibody fragments.

19. The protein conjugate according to claim 18, wherein the physiologically active polypeptide is human growth hormone, interferon-alpha, granulocyte colony stimulating factor, erythropoietin or a Fab' antibody

fragment.

20. A method for preparing the protein conjugate of claim 1, comprising:

(a) covalently linking one or more non-peptide polymers having a reactive group at both ends thereof, one or more physiologically active polypeptides and one or more immunoglobulin Fc fragments; and

(b) isolating the said protein conjugate essentially comprising the covalently linked physiologically active polypeptide, non-peptide polymer and immunoglobulin Fc fragment.

21. The method according to claim 20, wherein the step (a) comprises:

(a1) covalently linking an immunoglobulin Fc fragment or physiologically active polypeptide to one end of an activated non-peptide polymer;

(a2) isolating a complex comprising the immunoglobulin Fc fragment or physiologically active polypeptide linked to the non-peptide polymer from a resulting reaction mixture; and

(a3) covalently linking an immunoglobulin Fc fragment or physiologically active polypeptide to the other end of the non-peptide polymer of the isolated

complex to provide a protein conjugate comprising the immunoglobulin Fc fragment and the physiologically active polypeptide, which are linked to each end of the non-peptide polymer.

22. The method according to claim 21, wherein, at the step (a1), the physiologically active polypeptide and the non-peptide polymer are used at a reaction molar ratio of 1:1.25 to 1:5.

23. The method according to claim 21, wherein, at the step (a1), the immunoglobulin Fc fragment and the non-peptide polymer are used at a reaction molar ratio of 1:5 to 1:10.

24. The method according to claim 21, wherein, at the step (a3), the complex obtained at the step (a2) and the immunoglobulin Fc fragment or physiologically active polypeptide are used at a reaction molar ratio of 1:0.5 to 1:20.

25. The method according to claim 21, wherein the steps (a1) and (a3) are carried out in the presence of a reducing agent.

26. The method according to claim 25, wherein the

reducing agent is selected from the group consisting of sodium cyanoborohydride ($NaCNBH_3$), sodium borohydride, dimethylamine borate and pyridine borate.

27. A pharmaceutical composition for enhancing in vivo duration and stability of a physiologically active polypeptide, comprising the protein conjugate of claim 1 and a pharmaceutically acceptable carrier thereof.

28. The pharmaceutical composition according to claim 27, wherein the non-peptide polymer is covalently linked via a reactive group at both ends thereof to the physiologically active polypeptide and the immunoglobulin Fc fragment.

29. The pharmaceutical composition according to claim 28, wherein one or more complexes of the physiologically active polypeptide and the non-peptide polymer are covalently linked to a single molecule of the immunoglobulin Fc fragment.

30. The pharmaceutical composition according to claim 27, wherein the immunoglobulin Fc fragment is non-glycosylated.

31. The pharmaceutical composition according to claim

27, wherein the immunoglobulin Fc fragment is composed of one to four domains selected from the group consisting of C_H1, C_H2, C_H3 and C_H4 domains.

32. The pharmaceutical composition according to claim 31, wherein the immunoglobulin Fc fragment further includes a hinge region.

33. The pharmaceutical composition according to claim 27, wherein the immunoglobulin Fc fragment is selected from the group consisting of Fc fragments from IgG, IgA, IgD, IgE, IgM, and combinations and hybrids thereof.

34. The pharmaceutical composition according to claim 33, wherein the immunoglobulin Fc fragment is selected from the group consisting of Fc fragments from IgG1, IgG2, IgG3, IgG4, and combinations and hybrids thereof.

35. The pharmaceutical composition according to claim 34, wherein the immunoglobulin Fc fragment is an IgG4 Fc fragment.

36. The pharmaceutical composition according to claim 35, wherein the immunoglobulin Fc fragment is a human aglycosylated IgG4 Fc fragment.

37. The pharmaceutical composition according to claim 28, wherein the reactive group of the non-peptide polymer is selected from the group consisting of an aldehyde group, a propione aldehyde group, a butyl aldehyde group, a maleimide group and a succinimide derivative.

38. The pharmaceutical composition according to claim 37, wherein the succinimide derivative is succinimidyl propionate, succinimidyl carboxymethyl, hydroxy succinimidyl or succinimidyl carbonate.

39. The pharmaceutical composition according to claim 38, wherein the non-peptide polymer has a reactive aldehyde group as a reactive group at both ends thereof.

40. The pharmaceutical composition according to claim 27, wherein the non-peptide polymer is linked at each end thereof to a free reactive group at an amino terminal end, a lysine residue, a histidine residue or a cysteine residue of each of the immunoglobulin Fc fragment and the physiologically active polypeptide.

41. The pharmaceutical composition according to claim 27, wherein the non-peptide polymer is selected from the group consisting of polyethylene glycol single polymers, polypropylene glycol single polymers, ethylene glycol-

propylene glycol copolymers, polyoxyethylated polyols, polyvinyl alcohols, polysaccharides, dextrans, polyvinyl ethyl ethers, biodegradable polymers, lipid polymers, chitins, hyaluronic acids, and combinations thereof.

42. The pharmaceutical composition according to claim 41, wherein the non-peptide polymer is polyethylene glycol.

43. The pharmaceutical composition according to claim 27, wherein the physiologically active polypeptide is selected from the group consisting of hormones, cytokines, enzymes, antibodies, growth factors, transcription regulatory factors, coagulation factors, vaccines, structural proteins, ligand proteins and receptors.

44. The pharmaceutical composition according to claim 43, wherein the physiologically active polypeptide is selected from the group consisting of human growth hormone, growth hormone releasing hormone, growth hormone releasing peptide, interferons, interferon receptors, colony stimulating factors, glucagon-like peptides (e.g., GLP-1, etc.), G-protein-coupled receptor, interleukins, interleukin receptors, enzymes, interleukin binding proteins, cytokine binding proteins, macrophage activating factor, macrophage peptide, B cell factor, T cell factor, protein A, allergy inhibitor, cell necrosis glycoproteins,

immunotoxin, lymphotoxin, tumor necrosis factor, tumor suppressors, metastasis growth factor, alpha-1 antitrypsin, albumin, alpha-lactalbumin, apolipoprotein-E, erythropoietin, highly glycosylated erythropoietin, angiopoietins, hemoglobin, thrombin, thrombin receptor activating peptide, thrombomodulin, factor VII, factor VIIa, factor VIII, factor IX, factor XIII, plasminogen activating factor, fibrin-binding peptide, urokinase, streptokinase, hirudin, protein C, C-reactive protein, renin inhibitor, collagenase inhibitor, superoxide dismutase, leptin, platelet-derived growth factor, epithelial growth factor, epidermal growth factor, angiostatin, angiotensin, bone growth factor, bone stimulating protein, calcitonin, insulin, atriopeptin, cartilage inducing factor, elcatonin, connective tissue activating factor, tissue factor pathway inhibitor, follicle stimulating hormone, luteinizing hormone, luteinizing hormone releasing hormone, nerve growth factors, parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocortical hormone, glucagon, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, autotaxin, lactoferrin, myostatin, receptors, receptor antagonists, cell surface antigens, virus derived vaccine antigens, monoclonal antibodies, polyclonal antibodies, and antibody fragments.

45. The pharmaceutical composition according to claim 44, wherein the physiologically active polypeptide is human growth hormone, interferon-alpha, granulocyte colony stimulating factor, erythropoietin or a Fab' antibody fragment.